



WASHINGTON STATE PATROL

BIOCHEMICAL ANALYSIS PROCEDURES

CRIME LABORATORY DIVISION

June 2018

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Washington State Patrol Crime Laboratory Division
Biochemical Procedures

INTRODUCTION

The following procedural methods in biochemical analysis were developed through the efforts of the DNA Functional Area members in accordance with the Washington State Patrol Crime Laboratory Division Quality Manual, (WSPCLD-QM), Operations Manual, (WSPCLD-OM) and the DNA Quality Assurance Manual. It is not intended to be a complete treatise on biochemical methods available, but will serve as a reference on procedures approved for use by forensic scientists of the WSPCLD. Significant changes to any of the following procedures or additional procedures developed shall be discussed within the functional area. A copy of the proposed changes or new procedure needs to be provided in writing and/or in an electronic media format to all DNA functional area forensic scientists. A comment and revision period shall be given before any of the proposed changes are finalized in the manual. The final revised version requires DNA Technical Leader sign off, approval by management, and appropriate distribution according to WSPCLD policy.

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1.0 GENERAL EXAM PROCEDURES

1.0 GENERAL PRINCIPLES

Examinations of clothing (and similar exhibits) shall be conducted with the following standards:

- The examination shall be relevant.
- The examination shall be conducted in a manner consistent with current safety requirements.
- The examination shall be conducted in a manner which will preserve the integrity of the evidentiary value of the exhibit.
- The examination shall meet peer standards of thoroughness and documentation.

1.1 GENERAL KNOWLEDGE REQUIRED

In order to meet the goals listed above, the examiner shall be aware of:

- The potential significance of the presence or absence of various biological stains and of any or all of the following: directionality/sequence-of-deposit of staining; trace evidence (hairs, fibers, paint, glass, soil, etc.); cuts, tears, punctures; heat damage. Any such significance may vary from case to case; therefore, a thorough understanding of the alleged facts of the case will contribute to assuring the examination is relevant (sources of such alleged facts include police and physician reports, investigating officers, prosecutors, newspaper accounts, etc.). Consulting technical publications and/or other examiners may be necessary in order to evaluate the potential significance of some attributes of an exhibit.
- Current laboratory requirements and recommendations regarding the handling and disposal of biological samples.
- Current peer standards regarding case management and documentation of examinations. Common ways in which evidence may be contaminated or lost during the examination of an exhibit (and ways to prevent or minimize such contamination or loss).

1.2 GENERAL INSTRUCTIONS REGARDING RELEVANCE OF EXAMINATIONS

Relevance of examinations can be assured only if the management and documentation of a case is responsive to the premise that each case is or may be of a non-routine nature.

A fundamental aspect of case management is to identify and document the unique characteristics of the case and of any evidence that is associated with the case. The examiner shall evaluate which examinations, if any, are appropriate for each exhibit submitted to the laboratory/examiner. Equally important, the examiner shall evaluate whether additional exhibits should be submitted in order to ensure that, to the extent practically possible, all pertinent forensic evidence is developed.

The "Request for Laboratory Examination" form shall be carefully reviewed. Other available relevant documents, such as officer or physician reports or related earlier crime laboratory reports shall also be reviewed.

As needed and appropriate, the case facts and/or exhibits shall be discussed with knowledgeable individuals such as officers, prosecutor, and/or other examiners.

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1.3 GENERAL INSTRUCTIONS REGARDING SAFETY OF EXAMINATIONS

Biochemical and related examinations shall be carried out only by individuals having the equipment, supplies, and training (or close supervision) needed to ensure the safety of the examiner and his/her co-workers. All evidence shall be considered potentially hazardous and shall be handled accordingly.

Before any examination is begun, an examination area of an appropriate size and location shall be available for use, safety equipment and supplies shall be readily accessible, and the examiner and the examination area shall be appropriately protected from contamination or other harm.

The examination area shall be large enough that the examiner is unlikely to be injured because of the inordinate proximity of another worker. Required disposal containers, spill cleanup supplies, personal protective equipment (PPE), etc. shall be available and utilized when appropriate.

1.4 GENERAL INSTRUCTIONS REGARDING PRESERVING THE INTEGRITY OF EVIDENCE

Where there are alternative methods for storing, examining, and/or testing a particular exhibit, those methods which best ensure the integrity of the evidentiary value of the exhibit shall be utilized. Any exception shall be justified. Any actual or potential contamination, deterioration, or loss shall be documented.

The examination area shall be large enough to minimize the possibility of contamination of the evidence by other workers or other evidence.

The workbench/table shall be clean and uncluttered. Prior to the conducting of a clothing examination, the workbench/table shall be covered with paper which is larger overall than the garment which is to be examined. If the garment is larger than the available examination surface, the garment shall be examined in smaller sections. Each individually wrapped exhibit shall be examined on a new sheet of paper. Those exhibits packaged together in a single (innermost) wrapping may be examined on the same sheet of paper.

Evidence shall be handled as minimally as possible without compromising the thoroughness of the examination.

Collected samples, substrate controls, trace material, etc. shall be promptly documented, packaged, and labeled.

1.5 GENERAL INSTRUCTIONS REGARDING THOROUGHNESS OF EXAMINATIONS

The primary purpose of the clothing examination is to locate, document, and collect (as appropriate) biochemical stains of possible significance. The secondary purpose of the examination is to locate, document, and collect (when appropriate) any additional materials/characteristics having potentially significant evidentiary value. The examiner shall document and be prepared to justify any significant deviation from current peer standards. It is acknowledged that two examiners are unlikely to approach a case in identical manners and that current peer standards, in order to be practical, must be expressed and interpreted in an appropriately flexible manner.

1.6 GENERAL INSTRUCTIONS REGARDING DOCUMENTATION OF EXAMINATIONS

The recording of case notes shall be carried out in a manner which will contribute to an efficient and proper resumption of examinations after an interruption of any time length, allow a supervisor or peer to readily carry out a case review, and permit the examiner to appropriately prepare for a

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conference or testimony regarding the case. Evidence and evidence packaging shall be marked according to the regulations in the current versions of the WSPCLD-OM and DNA QA manuals.

Case notes shall include descriptions of evidence items. When appropriate, diagrams, sketches and/or digital images may also be utilized. Only the digital images printed in the case file shall be retained electronically.

Case notes shall be recorded in a manner consistent with the following guidelines:

- Each page of notes shall include the case number, analyst's initials, page number, and the date(s) of analysis.
- Evidence packaging shall be described.
- Evidence items shall be described as to type, identifying characteristics, and location(s) of stain(s), location(s) and approximate size(s) of cutting(s), etc.
- All tests (and their results) conducted as a part of the clothing examination shall be recorded. Lot numbers for chemical reagents utilized in testing shall also be recorded.

1.7 EXAMINATION PROTOCOL

The preceding general instructions shall be followed:

As the examination for biochemical stains is carried out, other potential evidence such as hair which is observed shall be documented and/or collected. After properly packaging any such collected evidence, it may be placed inside the package holding the item from which it was collected, or it may be packaged separately, given a unique identifier, and entered into evidence. Consult with the appropriate functional area section for procedural advice concerning other evidence observed. In the case of trace evidence regarding hair, depending on the nature of the investigation (i.e. investigator only interested in DNA) it may be appropriate for the DNA Forensic Scientist to evaluate the hair for DNA STR analysis suitability following the procedure outlined in Section 16 of this manual. Otherwise a more in depth microscopic analysis is available from the Microanalysis section.

An appropriately thorough examination may require inspecting the front, back, outside, and inside of the garment (pockets, cuffs, etc.) using the unaided eye, oblique lighting, backlighting, an ultraviolet light, an alternate light source, a hand-held lens, and/or a stereomicroscope. The testing and sampling of an item shall be based upon the training and experience of the analyst, taking into account the nature and quantity of available evidence, the request for analysis and the known facts of the case being examined. However, the analyst's decisions must pass the scrutiny of the peer review process.

Not all contents of a sexual assault kit may require testing (e.g. oral swabs, body swabs, underwear). The analyst will determine which contents require initial screening based on information provided at the time of submission.

After the examination is completed, items (whenever possible) shall be returned to the original packaging. The packaging shall then be sealed according to the regulations in the current versions of the WSPCLD-OM and DNA QA manuals.

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2.0 ALTERNATE LIGHT SOURCE

2.0 PURPOSE:

To screen an item of evidence for the presence of potential biological fluids when no stains are observed under normal lighting.

2.1 INTRODUCTION:

An Alternate Light Source (ALS) is a specialized light that combines powerful illumination with specific wavelengths. Physical evidence, such as fibers and biological fluids, may fluoresce or absorb when exposed to this type of light.

2.2 TESTING PROCEDURES:

- An examination using an ALS shall be conducted in a darkened room.
- Follow the manufacturer's recommendations for proper operation of the ALS.
- Select an appropriate wavelength and filter/goggles for the type of search being conducted.
- Spread out the item to be examined on clean paper and systematically scan the item for areas of fluorescence/absorption as appropriate.
- Mark and/or note any areas for further testing.

2.3 INTERPRETATIONS:

Fluorescence/absorption may indicate the presence of a biological fluid. However, further analysis (acid phosphatase test, sperm search, urea test, amylase, or Phenolphthalein test) must be performed before the presence of a biological fluid is determined. The absence of a fluorescence/absorption result does not confirm the absence of a biological fluid.

3.0 PHENOLPHTHALEIN TEST (MODIFIED KASTLE-MEYER TEST) PRESUMPTIVE TEST FOR BLOOD

3.0 PURPOSE:

To test for the presumptive presence of blood.

3.1 INTRODUCTION:

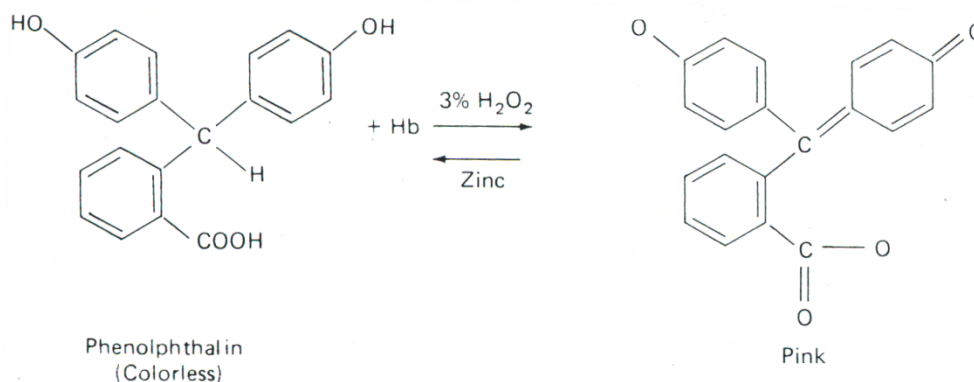
All catalytic blood tests depend upon an oxidation reaction in which an oxidant, hydrogen peroxide for example, oxidizes a colorless material to a colored one (e.g., phenolphthalin to phenolphthalein). Ionic iron forms cyclic chelate structures with many organic compounds, and often such iron-chelates possess catalytic activity in oxidation reactions. An example of such a biological catalyst is peroxidase, wherein a chelated metal decomposes hydrogen peroxide to form hydroxide ion and an oxy radical, which are ultimately converted to water and a metal-hydroxide ion complex:



Hemoglobin contains an iron-heme chelate complex which has demonstrated this particular behavior.

3.2 THE PHENOLPHTHALEIN TEST:

Phenolphthalin is an organic molecule which is oxidized by a compound with a reactive metal-oxygen bond commonly found in peroxidases. Prior to its use in this test, the phenolphthalein must be reduced to phenolphthalin. The reduced form is then used to test for the presence of the Fe(IV)=O species. In a positive test, formation of a pink color indicates the oxidation of phenolphthalin back to phenolphthalein (under basic conditions).



The phenolphthalein test has a maximum reported sensitivity for liquid blood of a 1:10,000,000 dilution and a maximum reported sensitivity on a dried blood stain of 1:10,000. While this test is highly sensitive to a minute trace of hemoglobin and its derivatives, it suffers from interference by other iron-heme containing materials, such as catalase, peroxidase, and cytochromes. Biological structures containing copper are also a noted strong interferent. In addition, strong oxidizing agents and metallic salts can also produce spurious reactions.

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3.3 TESTING PROCEDURES:

Before use on casework samples, working reagents shall be tested with a positive blood control each day of use. A negative control shall also be tested. The results of these tests shall be documented in case notes.

The test itself may be performed by one of the following methods:

3.3.1 IF THE STAIN IS VISIBLE:

The stain may be rubbed with a clean cotton swab (or filter paper) moistened with sterile dH₂O. Phenolphthalin reagent is then added to the cotton swab or the filter paper. After visual inspection, a similar amount of 3% hydrogen peroxide is applied to the test swab or paper.

A portion of the stain may be cut out or scraped off an object and placed into a spot well. Phenolphthalin reagent is then added to the spot well. After visual inspection, a similar amount of 3% hydrogen peroxide is added to the spot well.

3.3.2 IF THE STAIN IS NOT VISIBLE:

A general swab or mapping of the area may be performed. Phenolphthalin reagent is then added to the cotton swab or the filter paper. After visual inspection, a similar amount of 3% hydrogen peroxide is applied to the test swab or paper.

3.4 INTERPRETATIONS:

A color change to pink (within 5 seconds after adding 3% H₂O₂) indicates a positive reaction.

The phenolphthalein test is a presumptive test for blood. Careful consideration shall be given to positive reactions on/in certain substrates or substances (i.e. leather and feces). When a positive result is obtained, it is necessary to consider carefully whether the test result could have been given by something other than heme from blood. The specificity of various catalytic reagents has been studied extensively. A spurious reaction may be obtained from substances other than bloodstains. These substances may be conveniently divided into two groups:

3.4.1 CHEMICAL OXIDANTS AND CATALYSTS:

Copper and nickel salts most frequently show reactions.

Rust, formalin, potassium permanganate, potassium dichromate, some bleaches, hypochlorite, iodine, and lead oxides.

Oxidizing compounds such as copper, potassium ferricyanide, and nickel and cobalt nitrates, and some sulfocyanates.

The behavior of chemical oxidants is quite different from that of blood. Chemical oxidants will give a discoloration before the addition of the hydrogen peroxide. Therefore, a reaction for blood is distinguished by use of the two solution test procedure outlined above.

The color of the stain tested must be considered and the evidence of usual observation added to the intelligent use of this test. A stain on cloth of these salts strong enough to give a positive reaction will (a) discolor the cloth (unless the cloth is of identical color) and (b) show small crystals in the cloth where the salt has dried. The stain color will not be that of blood. Invisible traces of blood can be detected. Invisible traces of these salts are not detected using the method described.

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3.4.2 PLANT SOURCES:

Vegetable peroxidases are the most important class of substances which show false positive reaction with chemical color tests. The following plant tissues may react with the phenolphthalin reagent and be mistaken for blood: apple, apricot, bean, blackberry, Jerusalem artichoke, horseradish, potato, turnip, cabbage, onion, and dandelion root.

The color of the stain must be observed before any tests are done, and in most cases, this is not the color of blood staining. Green and white are the most common colors to find associated with plant materials. The plant peroxidases appear to reside in the particulate contents of the cells of the plant tissue, the juice of the plant giving a negative or only a faint positive reaction. A strong positive reaction could be associated with tissue or tissue fragments: such fragments are detectable and identifiable by microscopic examination.

As an alternative, heating the sample stain or extract to 100° C for five minutes will differentiate the plant peroxidases from blood sources, since heme is stable at this temperature, while plant peroxidases are rapidly deactivated.

Laboratory requests for after-the-fact crime scene reconstructions and/or bloodstain pattern analysis (interpretations) on submitted items conducted in the laboratory will require a disclaimer in the report indicating the assumptions made by the analyst concerning the stains.

3.5 **CONCLUSIONS:**

3.5.1 NEGATIVE REACTION:

No blood was detected (found).

3.5.2 POSITIVE REACTION (NO VISIBLE STAINING):

Indications of blood were detected (or the presumptive presence of blood was detected).

3.5.3 POSITIVE REACTION (VISIBLE STAINING):

Staining consistent with blood was detected (found).

3.6 REFERENCES:

Culliford BJ. (Phenolphthalein). In: The Examination and Typing of Bloodstains in the Crime Laboratory. U.S. Department of Justice, Law Enforcement Assistance Administration; 1971; p. 2-93 to 2-101.

Gaensslen RE. (Phenolphthalein). In: Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: United States Department of Justice; 1983; p. 101-16.

Higaki RS, Philp WM. A Study of the Sensitivity, Stability and Specificity of Phenolphthalein as an Indicator Test for Blood. Canadian Society of Forensic Science Journal 1976; 9 (3):97-102.

Lee HC. Identification and Grouping of Bloodstains In: Saferstein R, editor. Forensic Science Handbook.

Englewood Cliffs: Prentice Hall; 1982; p. 272-6.

Spalding RP, Cronin WF. Technical and Legal Aspects of Forensic Serology: A Laboratory Manual. Washington D.C.: U.S. Department of Justice, Federal Bureau of Investigation; 1984.

4.0 ABACARD® HEMATRACE® HUMAN BLOOD TEST

4.0 PURPOSE:

To qualitatively detect human hemoglobin (a component of blood) in forensic samples. This test shall only be conducted after a positive presumptive test for blood. There may be special exceptions where Hematrace is used as the sole independent test however the rationale to explain the testing approach used needs to be well documented in the notes and the conclusion and or remarks would need to be modified as appropriate to reflect the limitations of the testing performed. Prior approval (date and initials) of the DNA Supervisor shall be documented in the notes for these special exceptions. Due to possible reactivity with semen, this test should be used with caution on stains (suspected of) containing semen.

4.1 TESTING PROCEDURES:

1. Place a sample into a sterile microcentrifuge tube.

Note 1: For old stains, add approximately 15 µl of 1% ammonia solution to the sample in the tube. Incubate at room temperature for about 15 minutes, vortexing occasionally. Proceed to step two and then skip step three. Follow the procedure from step four.

Note 2: If the 37°C incubation described for sperm search is used to prepare an extract, skip to step 5.

2. Add approximately 200 µl of sterile dH₂O (or PBS) to the sample in the tube and vortex.
3. Incubate at room temperature for approximately 15 minutes, vortexing occasionally.
4. Place cutting in a spin basket and spin at maximum speed for 3 minutes.
5. Remove a portion of the supernatant. Do not disturb the pellet.
6. Ensure that the removed supernatant is then diluted until it appears colorless (no reddish color) and it is a minimum of 150 µl in volume.
7. The microcentrifuge tube with the pellet (and cutting) may be used for any extraction procedure.
8. Place the 150 µl of liquid on the sample well, well "S" of the HemaTrace® cassette.
9. The result shall be read at 10 minutes. A positive result may be seen earlier.

Note: The result shall not be read after 10 minutes since nonspecific reactions may result in a false positive.

4.2 INTERPRETATIONS:**4.2.1 POSITIVE HEMATRACE® RESULT:**

The formation of a visible pink line in the test “T” and control “C” regions.

4.2.2 NEGATIVE HEMATRACE® RESULT:

No formation of a visible pink line in the test “T” region, and a visible pink line in the control “C” region.

4.2.3 INCONCLUSIVE HEMATRACE® RESULT:

An incomplete or questionable visible pink line in the “T” region, and a visible pink line in the control “C” region.

4.2.4 INCONCLUSIVE TEST (FAILED HEMATRACE® CASSETTE):

No visible pink line in the control “C” region regardless of whether or not the test “T” region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Note: Interpretation of the result of the test shall be documented in the case file.

4.3 INTERPRETATION CAUTIONS:

Product literature reports that the lower limit of sensitivity for human hemoglobin is approximately 1 to 1,000,000 dilution of whole human blood.

A “High Dose Hook Effect” may be seen for extremely concentrated human hemoglobin samples (such as whole human blood) which appears as a negative result (no pink line in the test “T” region, pink line visible in the control “C” region). If such a sample is suspected, the supernatant should be retested using a 10 to 10,000 fold dilution.

It has been reported that ferret blood and upper primate blood can produce false positives with this product. A 1 to 10 dilution of blood from an orangutan tested negative during internal validation work.

An approximately 1 to 100 dilution of neat semen and a neat semen stain were both positive during internal validation work (a dilution of 1:1000 neat semen was negative). Therefore, this test should be used in conjunction with a presumptive test for the presence of blood to decrease the possibility of false positives with semen stains. The product literature suggests that the sensitivity of the product can detect trace levels of hemoglobin that might be present in other body fluid samples (e.g. urine, semen, feces, saliva, vaginal fluid, perspiration).

The reported sensitivity for the phenolphthalein test is approximately 10 times greater than the sensitivity of the HemaTrace® cassettes. Therefore, a weak positive phenolphthalein reaction may occur (or may be obtained) with a negative test using the HemaTrace® cassette with human blood.

An internal study indicated that materials treated with phenolphthalein reagents, when added to the HemaTrace® assay, may cause a false negative result. Therefore, the HemaTrace® test should not be run on samples which have been exposed to phenolphthalein reagents.

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4.4 CONCLUSIONS:**4.4.1 HEMATRACE® POSITIVE:**

Human blood was detected.

4.4.2 HEMATRACE® NEGATIVE:

Indications of blood were detected (or the presumptive presence of blood was detected), however human origin could not be confirmed.

Note: If a HemaTrace® negative result is obtained from analysis of a readily visible stain, the wording "non-human origin" is appropriate.

4.4.3 INCONCLUSIVE HEMATRACE® RESULT:

Indications of blood were detected (or the presumptive presence of blood was detected), however the test for human origin was inconclusive.

4.5 REFERENCES:

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Hochmeister MN. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. Journal of Forensic Sciences 1999; 44 (6):597-602.

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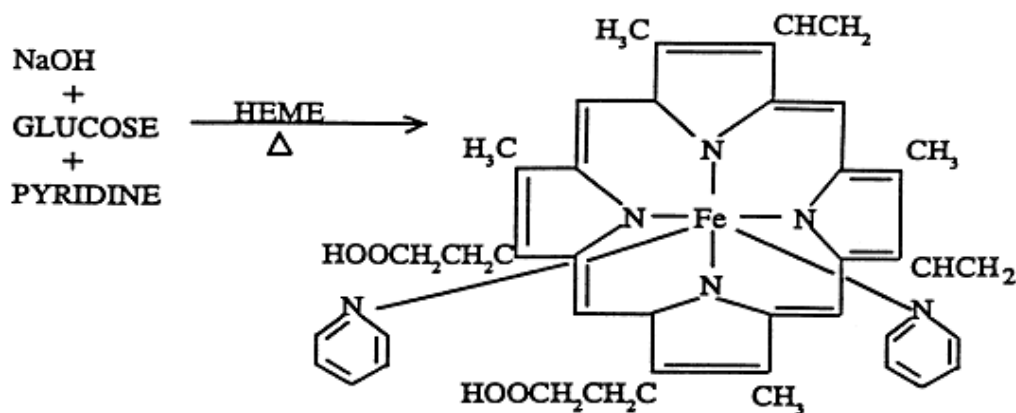
5.0 TAKAYAMA TEST (HEMOCHROMOGEN CRYSTAL TEST) THE IDENTIFICATION OF BLOOD

5.0 PURPOSE:

To confirm the presence of blood. When performed, the Takayama test is typically done following a positive visual examination and positive catalytic reaction.

5.1 INTRODUCTION:

Both ferroprotoporphyrin and ferriprotoporphyrin have the ability to combine with other nitrogen containing compounds in addition to globin. Such substances include other proteins, ammonia, cyanide, nicotine, and pyridine. The resultant products are called hemochromogens. The crystals may be formed at acid or alkaline pH; however, the Takayama reagent which is preferred by many is alkaline in nature.



Hemochromogen (Pyridineferroprotoporphyrin)

The hemochromogen crystals observed in a positive test come from a number of reactions involving the components of the reagent. Sodium hydroxide serves to affect an alkaline hydrolysis to liberate the heme prosthetic groups from the globin. The heme iron at this point is in the ferric (+3) state, due to the formation of methemoglobin on drying of the bloodstain. (The third positive charge of the iron is neutralized by a hydroxyl ion.) On heating, the ferric iron reduces to ferrous (+2) and the pyridine combines with it to form the insoluble crystalline product, hemochromogen or pyridineferroprotoporphyrin. The test is positive with as little as 0.001 ml of blood or 0.1 mg of hemoglobin and can often give positive results with blood removed from wood or leather surfaces.

5.2 TESTING PROCEDURES:

Before use on casework samples, the working reagent shall be tested with a positive blood control each day of use. A negative control shall also be tested. The results of these tests shall be documented in case notes.

The test itself is performed as follows:

1. Place a small portion of the suspected bloodstain (thread, scraping, cutting, extract, etc.) on a microscopic slide and cover with a cover slip.

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2. Let two drops of Takayama reagent flow under the cover slip. Two forms of pink/red crystals (needle-shaped or rhomboid) are formed in a few minutes and are observed microscopically.
3. When the reagent is freshly prepared, the reaction time is approximately 3-5 minutes. The reaction time decreases to a few seconds when the reagent is aged for 1-2 weeks. The reaction time may increase as the reagent ages longer.
4. Gentle heating (no flame) may be needed to help old, fixed stains react with the Takayama reagent.

Note: A smaller (broken) piece of cover slip can be used with a small amount of sample in order to increase detection.

5.3 INTERPRETATION:

The development of microscopic crystals (needle-shaped or rhomboid) formed within a few minutes of adding the Takayama reagent is a positive test. A complete crystallization may be difficult to obtain, especially with old blood samples. The formation of different types of crystals may also result.

Note: The failure to obtain a positive Takayama test does not necessarily indicate the absence of blood.

5.4 CONCLUSIONS:

5.4.1 TAKAYAMA POSITIVE:

Blood was detected.

5.4.2 TAKAYAMA NEGATIVE:

The presence of blood was not confirmed.

Note: this presumes that there was a positive phenolphthalein test otherwise it would read no blood was detected.

5.5 REFERENCES:

Gaensslen R. (Takayama). In: Sourcebook in Forensic Serology, Immunology and Biochemistry. U.S. Department of Justice; 1983; p. 85-7.

Lee HC. Identification and Grouping of Bloodstains. In: Saferstein R, editor, Forensic Science Handbook. Englewood Cliffs: Prentice Hall; 1982; p. 276-9.

Lehninger AL. Biochemistry. 2nd ed. New York: Worth Publishers; 1975.

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6.0 SPECIES OF ORIGIN DETERMINATION

6.0 PURPOSE:

To determine the species of origin of unknown staining in forensic samples.

6.1 INTRODUCTION:

The Ouchterlony test is the precipitin test used by the WSP Crime Laboratory.

6.2 IMMUNODIFFUSION TEST (OUCHTERLONY)

The Ouchterlony test involves the use of agar plates with wells for both antibodies and antigens. The two reactants diffuse into the gel where immunoprecipitates will form at the point of equivalence for each antigen-antibody pair. The site of the formation of the precipitin band depends on the diffusion coefficient of antigen and antibody and not on their relative concentrations. Each precipitate acts as an immunospecific barrier for the particular pair of reactants and prevents their further diffusion, but it does not hinder diffusion of other reactants. If two reactants are reasonably balanced, the precipitate does not migrate any further, but grows peripherally in lines or arcs at constant angles to the line joining the two wells. However, in an unbalanced mixture, the so-called Liesegang phenomenon or formation of multiple precipitin bands may occur.

6.3 TESTING PROCEDURES:

1. Place a sample into a sterile microcentrifuge tube. Add PBS or sterile dH₂O. Incubate at room temperature and dilute (if necessary) to yield a "straw" color. (A substrate control, if appropriate, is extracted and diluted the same as the stain.)

Note: For old stains, add approximately 15 µl of 5% NH₄OH to the sample in the tube. (For weak stains, 5% NH₄OH and 0.05M DTT have also been found to be good for extraction.)

2. Briefly centrifuge sample. A spin basket may be used to maximize recovery.
3. Pipet 10-15 µl of appropriate antibody into the center well of the gel. Pipet 10-15 µl of the stain and control extracts into the outside wells. (The reverse loading scheme may also be used to test for multiple species simultaneously). A diluent blank (e.g., PBS, dH₂O) shall be included as a negative control. A substrate sample may be included on the run. The inclusion of a control for the type of animal serum in which the antiserum was prepared may be run at the option of the analyst.
4. Cover the plate and allow to stand at room temperature in a moisture chamber overnight.
5. The sample may be centrifuged to pellet the material for use in an extraction.
6. Indirect light (or backlighting) may be used to observe the precipitin lines between the wells, indicating a positive test. If necessary, proceed with the staining procedure to enhance the visibility of the precipitin lines.
7. Results shall be documented in the case file.

Note: Positive and negative controls shall always be run in conjunction with the questioned samples.

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6.3.1 COOMASSIE STAINING METHOD:

1. Remove gel from the plate and place on hydrophilic side of GelBond™.
2. Press gel with moistened filter paper (Whatman® #1 is recommended) on top of gel. Add blotting paper and weight on top.
3. Press gel until dry (approximately 30 minutes).
4. Wash in 1M NaCl to remove unprecipitated protein (several hours to overnight).
5. Wash approximately 5 minutes in dH₂O (longer may dissolve Ag-Ab complex).
6. Repeat steps 2 and 3. (Optional Step)
7. Use an appropriate method to dry the gel.
8. Stain with Coomassie Blue for approximately 10 minutes.
9. Destain with Destain Solution until background clears.

6.4 INTERPRETATIONS:

The presence of precipitin lines/bands between the wells indicates a positive test.

6.5 CONCLUSIONS:**6.5.1 POSITIVE PRECIPITIN BAND:**

The presence of (blood/tissue/substance) of (name appropriate taxonomic classification for the specificity of anti-serum used) origin was identified.

6.5.2 NO PRECIPITIN BANDS OBSERVED:

The origin of the (tested material) could not be determined.

Note: If no precipitin bands are observed from analysis of a readily visible blood stain, the wording "non-human origin" is appropriate (providing one of the antisera used was anti-human or a negative Hematrace result was also obtained).

6.5.3 POSITIVE AND/OR NEGATIVE CONTROL FAILURE (INSUFFICIENT SAMPLE TO RERUN TEST):

The tests results were inconclusive.

6.6 REFERENCES:

Culliford BJ. (Species). In: The Examination and Typing of Bloodstains in the Crime Laboratory. Washington, D.C: U.S. Department of Justice, Law Enforcement Assistance Administration; 1971; p. 2-93-2-101.

Gaensslen RE. (Species). In: Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: U.S. Department of Justice; 1983; p. 221-41.

Lee HC. Identification and Grouping of Bloodstains. In: Forensic Science Handbook. Englewood Cliffs: Prentice Hall; 1982; p. 283-97.

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Sensabaugh GF. Molecular Evolution and the Immunological Determination of Species. International Microform Journal of Legal Medicine 1976 Article 219; 11 (2).

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7.0 ACID PHOSPHATASE TEST

7.0 PURPOSE:

To screen for the presence of semen in forensic samples by testing for acid phosphatase activity.

7.1 INTRODUCTION:

Acid phosphatase (AP) is an enzyme found at elevated levels in semen. When the Brentamine reagent is applied to the test sample, a purple color forms in the presence of AP. The AP enzyme cleaves phosphate from sodium α -naphthyl phosphate, freeing naphthol. The freed naphthol then couples with buffered Fast Blue B resulting in a purple azo dye.



7.2 TESTING PROCEDURES:

Before use on casework samples, working reagents shall be tested with at least a 1:100 diluted positive semen control each day of use. A negative control shall also be tested. The results of these tests shall be documented in the case notes.

Note: It is recommended to prepare the diluted positive semen control with PBS (instead of water) to increase its long-term reactivity if stored frozen and used after a long period of time.

The test itself may be performed by one of the following methods:

7.2.1 SPOT TEST (INDIVIDUAL STAINS):

1. Moisten a piece of filter paper or swab with dH₂O.
2. Press or gently rub the filter paper or swab on the suspected semen stain.
3. Apply Brentamine reagent to the paper or swab and allow 2-3 minutes for color development.
4. Test result and approximate location(s) of stain(s) shall be documented in the case file.

7.2.2 MAPPING (WHOLE GARMENTS, BED SHEETS, UNDERWEAR CROTCH PANELS, ETC.):

1. Cut filter paper to appropriate lengths. Moisten the filter paper with dH₂O using a spray bottle and blot.
2. Lay the moist filter paper over the area of interest and press onto the item for a minimum of 30-60 seconds.

Note: Additional filter paper, a glass or plastic plate, and weight (i.e., books or bricks) on the paper may be useful at this step. Large areas may require two or more filter paper overlays laid side by side to map efficiently. If multiple sheets are used, they should be marked in a manner to facilitate their orientation to one another and to relate positive test reactions back to specific areas of the evidence item.

3. After pressing, hang the filter paper in a chemical fume hood and spray with Brentamine reagent and allow 2-3 minutes for color development.

Note: Brentamine reagent may also be applied drop-wise to the area to avoid aerosolizing the reagent. The drop-wise application does not require a chemical fume hood.

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4. Test result and approximate location(s) of stain(s) shall be documented in the case file.

7.3 INTERPRETATIONS:

Semen stains should give a fast (immediate to within three minutes) purple color reaction depending on the amount of AP present. Slower developing color reaction may be due to a weak semen stain or from AP in other body fluids.

7.4 INTERPRETATION CAUTIONS:

1. This test is a presumptive test only. Positive reactions should be confirmed by the ABACard® p30 test or a microscopic sperm search. Slow or weak reactions and different color reactions may not be seminal fluid.
2. The enzyme acid phosphatase occurs in other body fluids, bacteria, fungi, and many plants. Common weak reactions may be seen in:
3. Vaginal secretions (especially during pregnancy or with bacterial infection). Generally, the reaction is slow and the resulting color is faint. However some females may have higher endogenous levels of AP activity that may provide stronger results.
4. Fecal stains. The reaction is slow and the resulting color is faint pink.
5. Certain plants and fungi.

7.5 CONCLUSIONS:

Please refer to 9.5 for conclusions referring to any tests for the presence of semen.

7.5.1 POSITIVE REACTION (P30 NEGATIVE, NO SPERM DETECTED WITH MICROSCOPIC SEARCH):

No semen was detected.

(or depending on the nature of the evidence, i.e. strong result from an intimate swab from female child and male DNA was identified)

Indications of (or the apparent presence of) semen were detected (found) but could not be confirmed.

7.5.2 NEGATIVE REACTION (P30 NOT TESTED, NO MICROSCOPIC SPERM SEARCH PERFORMED):

No indications of semen were detected (found).

7.6 REFERENCES:

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor, Forensic Science Handbook Vol. 2. Englewood Cliffs Prentice Hall; 1988; p. 347-55.

Davies A, Wilson E. The Persistence of Seminal Constituents in the Human Vagina. Forensic Science 1974; 3 (1):45-55.

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Metropolitan Police Forensic Science Laboratory. (AP). In: Biology Methods Manual. London: Commissioner of Police of the Metropolis; 1978; p. 3-17 to 3-9.

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8.0 SPERM SEARCH / CHRISTMAS TREE STAIN

8.0 PURPOSE:

To isolate and identify spermatozoa and other cellular components (if present) through microscopy and staining techniques.

8.1 INTRODUCTION:

Utilizing this staining procedure, coupled with the microscopy techniques, sperm cells can be reliably identified and differentiated from other cellular material/components. The nuclear material of the cell is stained red, while the cellular membranes are stained green. Sperm heads are usually well differentiated with the acrosomal cap unstained or lightly stained and the posterior (nuclear) portion more darkly stained. This color staining is augmented by phase contrast microscopy in which the staining appears reversed, with the acrosomal cap appearing darker than the posterior (nuclear) region.

8.2 TESTING PROCEDURES:

1. Remove a portion of the stain, stained material, or vaginal aspirate and place into an appropriate microcentrifuge tube.

Notes:

- If an unstained smear slide was received as a case sample, proceed to step 9.
 - If an appropriately stained smear slide without a cover slip was received as a case sample, proceed to step 13.
2. Add 100-1000µl of sterile PBS to each microcentrifuge tube and incubate at 37° C for a minimum of 30 minutes.
 3. Following incubation, pulse spin the sample(s). Transfer the substrate material into a spin basket insert and place the basket into the tube containing the stain extract.
 4. Centrifuge for 5 minutes (at top speed or as appropriate).
 5. Remove the basket insert with the substrate material from the extract tube and set aside.
 6. While being careful not to disturb the pellet in the microcentrifuge tube, remove most of the supernatant fluid (leaving approximately 20-50µl) and transfer the supernatant into a separate labeled sterile 1.5 ml tube.

Note: The transferred supernatant may be used for PBS extraction compatible serological tests which includes p30, and amylase. If the sample supernatant is not used immediately, it may be frozen for later use.

7. Resuspend the cell pellet in the remaining fluid in the microcentrifuge tube and place 3 µl of the suspended material on a microscope slide. (In some circumstances it may not be necessary to resuspend the pellet.)

Note: The extract and substrate may be taken forward for further DNA extraction procedures. The extracts shall be stored under appropriate conditions until further testing is performed.

8. Heat fix the sample to the microscope slide (a Bunsen burner, small torch, hot plate, or oven may be utilized).

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9. Apply Christmas Tree Stain A (red, nuclear fast red) to the heat-fixed cells on the microscope slide and allow stain to remain on the sample for 10-15 minutes.
10. Gently rinse the slide with dH₂O.
11. Apply Christmas Tree Stain B (green, picro indigo carmine) to the sample on the microscope slide and allow the stain to remain on the sample for 5-10 seconds.
12. Gently rinse the slide with ethanol and allow the slide to dry.
13. Using permount (or another mounting medium), apply a coverslip to the slide.
14. Conduct a systematic search of the slide using a microscope which has phase contrast capabilities between 200X to 400X magnification.

Notes: If no spermatozoa are observed on the created slide, proceed with testing for p30.

If low spermatozoa are observed and/or weak p30 results are obtained, additional sample may be removed and combined with the original substrate, and a differential slide may be made (see STR Analysis Procedure Differential Lysis for Semen Stains steps 7-15).

NOTE: The analyst can choose to delay the micro exam for sperm as described in step 6 of the STR Casework Procedures Manual until after the sperm wash in step 12 or step 13. This can only be done during the examination of oral swabs and genital/anal swabs collected for sexual assaults. A second exam after step 13 is optional.

8.3 INTERPRETATIONS:

Case notes shall document whether spermatozoa were observed or not, the relative number of spermatozoa observed, any unusual characteristics the spermatozoa exhibit (pyriform heads, tail defects, etc.), other cellular and/or non-cellular materials observed to include identity (or general characteristic) and relative number. As appropriate, the relative location of spermatozoa on the slide shall be documented in the case notes.

8.4 CONCLUSIONS:

Please refer to 9.5 for conclusions referring to any tests for the presence of semen.

8.4.1 SPERMATOOZOA OBSERVED MICROSCOPICALLY:

Semen was identified (detected).

8.4.2 NO SPERMATOOZOA OBSERVED MICROSCOPICALLY (P30 TEST POSITIVE, AP-POSITIVE):

Semen was identified (detected).

8.4.3 NO SPERMATOOZOA OBSERVED MICROSCOPICALLY (P30 TEST NEGATIVE, AP-POSITIVE):

No semen was detected

(or)

Indications of (or the apparent presence of) semen were detected (found) but could not be confirmed.

8.4.4 NO SPERMATOOZOA OBSERVED MICROSCOPICALLY (P30 TEST NEGATIVE, AP-NEGATIVE):

No semen was detected.

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8.4.5 NO SPERMATOZOA OBSERVED MICROSCOPICALLY (NO OTHER TESTING):

No spermatozoa were observed.

8.5 REFERENCES:

Allard JE. The Collection of data from finding in cases of Sexual Assault and the Significance of Spermatozoa on Vaginal, Anal, and Oral Swabs. Science and Justice 1997; 37 (2):99-108.

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor, Forensic Science Handbook Vol II. Englewood Cliffs: Prentice Hall 1988 p. 364-8.

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Davies A, Wilson E. The Persistence of Seminal Constituents in the Human Vagina. Forensic Science 1974; 3 (1):45-55.

Enos WF, Beyer JC. Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims. Journal of Forensic Sciences 1978; 23 (1):231-3.

Oppitz E. A New Color Method for Proof of Sperm in Moral Crimes. Arkiv Fur Krimin 1969; 144:145-8.

Serological Research Institute. "A Gram Modified Christmas Tree Stain" procedure. In: Serological Research Institute Methods Manual Vol. Document No. MM II-C, Revision No. 2; 2002 p. 1-2.

Willott GM, Allard JE. Spermatozoa – Their Persistence After Sexual Intercourse. Forensic Science International 1982; 19 (2):135-54.

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9.0 ABACARD® P30 SEMEN TEST

9.0 PURPOSE:

To test for the presence of semen by detecting p30 in forensic samples.

9.1 INTRODUCTION:

p30 is a protein that is used in the detection of semen. The intent of this test is to detect semen in samples with no/low levels of spermatozoa observed in a microscopic exam.

9.2 TESTING PROCEDURES:

1. Apply 200 µl of room temperature supernatant from the test sample to the sample well "S" on the ABACard® for p30. Maintain at room temperature.

Note: The supernatant used is from the PBS extraction step of the Sperm Search section of this manual. A dilution of the supernatant may be needed to yield 200 µl of liquid.

2. The result shall be read at 10 minutes. A positive result may be seen earlier.

Note: The result shall not be read after 10 minutes since nonspecific reactions may occur and may result in a false positive.

9.3 INTERPRETATIONS:

9.3.1 POSITIVE P30 RESULT:

The formation of a visible pink line in the test "T" and control "C" regions.

9.3.2 NEGATIVE P30 RESULT:

No formation of a visible pink line in the test "T" region, and a visible pink line in the control "C" region.

9.3.3 INCONCLUSIVE P30 RESULT:

An incomplete or questionable visible pink line in the "T" region and a visible pink line in the control "C" region.

9.3.4 INCONCLUSIVE TEST (FAILED CASSETTE):

No visible pink line in the control "C" region regardless of whether or not the test "T" region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Note: Interpretation of the result of the test shall be documented in the case file.

9.4 INTERPRETATION CAUTIONS:

Product literature reports that the lower limit of sensitivity for p30 detection is 4 ng/ml. A few studies have shown that a very small minority of women may have endogenous levels of p30 close to the minimum detectable level of the ABACard® assay. Therefore, caution should be used in interpreting lines that develop close to the 10 minute time limit of this test. The strength of the acid phosphatase result could also be a factor considered.

A "High Dose Hook Effect" may be seen for extremely concentrated p30 samples (such as neat semen on an item) which appears as a negative result (no pink line in the test "T" region, pink line

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visible in the control "C" region). If such a sample is suspected, the supernatant should be retested using a 10 to 10,000 fold dilution.

Internal validation work on this procedure reveals that a PBS (rather than dH₂O) extract is more stable over time. In addition, extracted samples should be run immediately or stored frozen and run within approximately 72 hours.

Concentrated male urine (approximating neat urine) may give a positive result.

The use of a substrate sample to aid in interpretation may be appropriate for some types of evidence.

False positive test results have been reported from recently deceased and decomposed male post-mortem anal swabs.

9.5 CONCLUSIONS:

9.5.1 SPERMATOOZA OBSERVED MICROSCOPICALLY:

Semen was detected/found.

9.5.2 POSITIVE P30 RESULT (NO SPERMATOOZA OBSERVED MICROSCOPICALLY, AP-POSITIVE):

Semen was detected/found.

9.5.3 POSITIVE P30 RESULT (NO SPERMATOOZA OBSERVED MICROSCOPICALLY, AP-NEGATIVE):

An indication of semen was detected/found.

9.5.4 NEGATIVE P30 RESULT (NO SPERMATOOZA OBSERVED MICROSCOPICALLY, AP-POSITIVE):

An indication of semen was detected but could not be confirmed.

9.5.5 NEGATIVE P30 RESULT (NO SPERMATOOZA OBSERVED MICROSCOPICALLY, AP-NEGATIVE):

No semen was detected.

9.5.6 POSITIVE P30 RESULT (SAK2 WORKFLOW-NO MICROSCOPIC EXAM):

An indication of semen was detected/found.

Also, add a note in the Remarks or Methods and Observations section: "Microscopic evaluation for the presence of spermatozoa was not performed on these samples. This analysis may be performed if requested".

9.5.7 POSITIVE P30 RESULT (SAK2 WORKFLOW-NO MICROSCOPIC EXAM):

The p30 test result obtained indicates the presence of semen.

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Also, add a note in the Remarks or Methods and Observations section: "Microscopic evaluation for the presence of spermatozoa was not performed on these samples. This analysis may be performed if requested"

9.5.8 NEGATIVE P30 RESULT (SAK2 WORKFLOW-NO MICROSCOPIC EXAM):

No indication of semen was detected.

Also, add a note in the Remarks or Methods and Observations section: "Microscopic evaluation for the presence of spermatozoa was not performed on these samples."

9.5.9 INCONCLUSIVE P30 RESULT: (FAILED C RESULT, NO OTHER TESTING):

No semen was detected.

9.5.10 INCONCLUSIVE P30 RESULT: (NO SPERMATOZOA OBSERVED MICROSCOPICALLY, AP-NEGATIVE): FS4/FS5 APPROVAL REQUIRED.

No semen was detected.

9.5.11 NEGATIVE AP: (NO OTHER TESTING):

No indication of semen was detected.

9.6 REFERENCES:

Abacus p30 Test Technical Information Sheet ABACard® p30 Test For The Forensic Identification of Semen. Abacus Diagnostics Inc; 2005.

Baechtel F. The Identification and Individualization of Semen Stains. In: Saferstein R, editor, Forensic Science Handbook Vol II. Englewood Cliffs Prentice Hall 1988; p. 355-60.

Denison SJ. Positive prostate- specific antigen (PSA) results in semen-free samples. Canadian Society of Forensic Science Journal 2004; 37 (4):197-206.

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Sippel, H, Lunetta, L., Positive prostate-specific antigen (PSA) reaction in rectal samples from deceased males. Promega International Symposium on Human Identification, October 2004.

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10.0 PHADEBAS® PAPER AMYLASE DIFFUSION

10.0 PURPOSE:

The Phadebas® paper amylase diffusion method can be useful in determining the presence of α -amylase in a variety of body fluid stains and swabs, which could indicate the presence of saliva.

10.1 INTRODUCTION:

Phadebas® has a water-insoluble starch polymer that carries a blue dye. If α -amylase is present, α -amylase hydrolyzes the starch polymer to form water-soluble blue fragments. The Phadebas® paper amylase diffusion method can be useful in determining the presence of α -amylase in stains and swabs, which could indicate the presence of saliva.

10.2 TESTING PROCEDURES:

1. Spray sterile distilled water liberally onto the back (non-blue side) of the Phadebas® paper. Be sure the paper is wet enough so it will not dry out during the testing process, but is not too wet.
2. Dampen item test area with sterile distilled* water using a spray bottle. Alternatively, the non-blue side (back) of the Phadebas® paper can be dampened again after placement on the test area.
3. Place the dampened piece of Phadebas® paper, blue side down on the desired test area.
4. Place 2-3 layers of butcher paper over the Phadebas® paper and apply light weight to ensure continual contact.
5. Allow contact to occur at room temperature for a maximum of 40 minutes or until a positive reaction is observed.

After recording the results, the Phadebas® paper may be tested for acid phosphatase activity (AP) by applying Brentamine reagent to the paper and allow 2-3 minutes for color development.

Note: If the Phadebas® paper is further tested for AP, a positive 1:100 semen control shall be run successfully in conjunction with, or on each day of testing.

*Note – using deionized water instead of distilled water can result in reduced sensitivity for the AP test.

10.3 INTERPRETATIONS:

Areas of a uniform blue color on the non-reagent side of the paper are indicative of the presence of α -amylase.

Note: The dye cleaved by amylase activity diffuses to the back white-side and the blue side becomes faded.

10.3.1 INTERPRETATION CAUTION:

α -Amylase activity is also reportedly found in semen, sweat, vaginal fluid, urine and fecal stains.

10.4 CONCLUSIONS:**10.4.1 UNIFORM BLUE COLOR ON NON-REAGENT SIDE:**

Indications of saliva were detected. (A remark may be added to the report that other body fluid sources cannot be eliminated – see interpretation cautions.)

10.4.2 NO BLUE COLOR ON NON-REAGENT SIDE:

No indications of saliva were detected

Note: For semen interpretations, cautions, and conclusions, see Acid Phosphatase section.

10.5 REFERENCES:

Willott GM. An Improved Test for the Detection of Salivary Amylase in Stains. Journal - Forensic Science Society 1974; 14 (4):341-4.

Willott GM, Griffiths M. A new method for locating saliva stains – spotty paper for spotting spit. Forensic Science International 1980; 15 (1):79-83.

<http://www.phadebas.com/>

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11.0 RAPID STAIN IDENTIFICATION OF HUMAN SALIVA (RSID®) CARDS

11.0 PURPOSE:

To screen for the presence of saliva by detecting human alpha-amylase in forensic samples.

11.1 INTRODUCTION:

The RSID® Saliva cards can be used to determine the presence of amylase in vaginal swabs, semen stains, and other body fluid stains that could indicate the presence of saliva.

11.2 TESTING PROCEDURES:

1. Combine 20 µL of room temperature supernatant (PBS or dH₂O) from the test sample with 80 µL of room temperature TBS+ Running Buffer or Universal Buffer provided in the RSID® Saliva kit.
2. Apply the 100 µL total volume prepared in Step 1 to the sample well "S" on the RSID® Saliva test card. Maintain at room temperature.
3. The result shall be read at 10 minutes. A positive result may be seen earlier.

11.3 INTERPRETATIONS:

11.3.1 POSITIVE AMYLASE RESULT:

The formation of a visible pink line in the test "T" and control "C" regions.

11.3.2 NEGATIVE AMYLASE RESULT:

No formation of a visible pink line in the test "T" region, and a visible pink line in the control "C" region.

11.3.3 INCONCLUSIVE AMYLASE RESULT:

An incomplete or questionable visible pink line in the "T" region, and a visible pink line in the control "C" region.

11.3.4 INCONCLUSIVE TEST (FAILED CASSETTE):

No visible line in the control "C" region regardless of whether or not the test "T" region exhibits a visible pink line. The test sample should be rerun, if appropriate and possible.

Notes: Interpretation of the result of the test shall be documented in the case file.

11.4 INTERPRETATION CAUTION:

Concentrated neat urine, feces, and breast milk may give a positive result close to the 10 minute time limit of this test.

Internal validation work on this procedure reveals that the test is more sensitive when a PBS (rather than dH₂O) extract is used.

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11.5 CONCLUSIONS:

11.5.1 POSITIVE:

An indication of human saliva was detected. (A remark may be added to the report that other body fluid sources cannot be eliminated – see interpretation cautions.)

(or)

Human amylase consistent with saliva was detected. (A remark may be added to the report that other body fluid sources cannot be eliminated – see interpretation cautions.)

11.5.2 NEGATIVE:

No indications of saliva were detected.

12.0 IDENTIFICATION OF FECAL MATERIAL

12.0 PURPOSE:

To characterize fecal material using a series of testing procedures.

12.1 INTRODUCTION:

Occasionally the need for forensic identification of fecal material on physical evidence is encountered. The presence of fecal material can be determined by evaluating the macroscopic and microscopic features as well as the chemical properties of the stain.

12.2 TESTING PROCEDURES:

12.2.1 SUSPECTED FECAL STAIN SAMPLE PREPARATION:

Prepare sample(s) as follows for the analytical tests:

1. An aqueous suspension is prepared by mixing a small portion of the questioned substance with 5-6 drops of dH₂O in a 1.5 ml microcentrifuge tube. To have enough sample for all tests, more than one suspension may need to be made.
2. Centrifuge the sample to form a pellet for those tests requiring a supernatant/pellet.

12.3 MACROSCOPIC EXAMINATION: COLOR AND ODOR

Fecal material is usually brown in color. Fecal material may be green, black, or red depending on diet, drugs and pathological conditions.

There is a distinctive odor associated with feces and this can be documented in the notes if detected.

12.4 ORIGIN DETERMINATION TESTING

Species origin may be determined by testing a portion of the above supernatant using either the Crossover Electrophoresis or Ouchterlony technique, or by extracting a sample for its DNA content and performing a human DNA quantification.

12.5 UROBILINOGEN DETERMINATION TESTING

This test relies on the formation of a fluorescent green zinc-urobilinogen complex. Urobilinogen is oxidized to urobilin which is soluble in alcohol. In the presence of neutral alcoholic zinc salts, the green complex is formed. Urobilinogen may be absent in stools from infants under 6 months.

12.5.1 UROBILINOGEN TEST PROCEDURES:

1. Pipet 3 drops of questioned aqueous sample material into a labeled 10 mm glass test tube.
2. Pipet 3 drops of a known human fecal material suspension (as a positive control) into a labeled 10 mm glass test tube.
3. Pipet 3 drops of dH₂O (as a negative control) into a labeled 10 mm glass test tube.
4. Add 3 drops of alcoholic mercuric chloride (HAZMAT) to each test tube.

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5. Add 3 drops of alcoholic zinc chloride (HAZMAT) to each test tube.
6. Examine the liquids in each tube under UV light (about 366 nm) and compare color to the known sample.

12.6 MICROSCOPIC EXAMINATION

1. Pipet one drop of the centrifuged pellet onto a glass slide and cover with a coverslip. Observe at 100X-400X for the following:
2. Bacteria and yeast.
3. Body cells derived primarily from the intestine.
4. Plant cells: spiral thickenings or fibers with pit, double contoured cells, plant hairs.
5. Muscle fibers: fibers with striations are only visible in undigested meat.
6. Starch grains.

12.7 INTERPRETATIONS:

12.7.1 UROBILINOGEN INTERPRETATION:

The presence of a stable, apple green fluorescence during examination with the UV light confirms that urobilinogen is present.

12.7.2 MICROSCOPIC EXAMINATION INTERPRETATION:

The presence of some of the microscopic elements is expected when fecal material is present.

12.8 CONCLUSIONS:

12.8.1 POSITIVE FOR HUMAN PROTEIN OR HUMAN DNA, AND UROBILINOGEN ALONG WITH THE OBSERVATION OF SOME MICROSCOPIC ELEMENTS:

The (sample tested) is consistent with human feces.

12.8.2 POSITIVE FOR UROBILINOGEN ALONG WITH THE OBSERVATION OF SOME MICROSCOPIC ELEMENTS:

The (sample tested) is consistent with feces.

12.8.3 NEGATIVE FOR UROBILINOGEN (AND/OR) NO MICROSCOPIC ELEMENTS OBSERVED:

The (sample tested) is not consistent with feces.

12.9 REFERENCES:

Gaensslen RE. In: Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: U.S. Department of Justice 1983; p. 197-8.

Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. London: Commissioner of Police of the Metropolis; 1978.

US Department of Justice. Federal Bureau of Investigation Laboratory. Serology Unit Protocol Manual. 1989.

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13.0 URINE

13.0 PURPOSE:

To presumptively identify urine and urine stains based on physical characteristics (odor and pH) and the chemical constituents THP and/or urea.

UREA DETECTION BY RADIAL DIFFUSION

13.1 INTRODUCTION:

The presence of urea in suspected urine stains may be detected in gels by radial diffusion of the products of enzymatic digestion by urease (carbonic acid and ammonia). The presence of a pH indicator in the gel results in a blue color development in diffusion zones where the enzyme has digested the urea.

13.2 TESTING PROCEDURES:

1. Extract the stain and substrate control cuttings (if applicable) in a minimum volume of dH₂O at room temperature or 37° for at least 30 minutes.

Note: Choice of an extractant other than dH₂O that has a basic pH will cause an immediate color change around the well, but this will usually dissipate in a few minutes.

2. Prepare gels (please see Reagent Preparation Section).
3. If desired, transfer the substrate material into a spin basket insert and place the basket into the tube containing the stain extract and centrifuge for 3-5 minutes at maximum speed.
4. Add 2 µl of supernatant from each sample and control extracts to corresponding wells on both test and control gel plates.

Note: Controls shall include an extractant (dH₂O) control, an extract of a known urine stain, and a substrate control from the fabric being tested (if used).

5. Allow diffusion to occur for ten minutes at room temperature. The diffusion will continue until the plate is unreadable, so it shall be read at ten minutes.

13.3 INTERPRETATIONS:

The appearance of a royal blue diffusion zone of at least 5 mm around a well on the test gel plate, with no corresponding color change on the control test plate, indicates the presence of urea by urease activity. The positive control sample shall show at least a 5 mm blue diffusion ring on the test gel plate or the test shall be rerun.

Interpretation Cautions:

Development of a blue color in the test gel plate as well as in the corresponding well of the control gel plate invalidates the test, indicating that something other than urea is responsible for the color change.

Bedrosian et. al., 1984, reports testing vaginal secretions, perspiration and tears. Perspiration gave a weak reaction (up to 4 mm in diameter on the test plate), due to the presence of small amounts of urea. Vaginal secretions and tears were negative.

In a dilution series on fresh urine, the test strongly detected urea at a 1:100 dilution of urine, and weakly detected it at a 1:200 dilution.

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Results are read qualitatively, although the assay has been shown to be sensitive to 0.078 µg/µl urea.

RSID™ URINE TO DETECT THP

13.4 INTRODUCTION:

The RSID™-Urine cards can be used to determine the presence of Tamm-Horsfall Protein (THP), which is the most abundant protein present in urine.

13.5 TESTING PROCEDURES:

1. Extract a sample in a minimal amount of RSID™-Urine Buffer (ex. 300 µL) for at least 30 minutes (and up to overnight) at room temperature or 37°C.
2. Apply 100 µL of the supernatant to the sample window of the cassette.
3. The result shall be read at 15 minutes. A positive result may be seen earlier.

13.6 INTERPRETATIONS:

13.6.1 POSITIVE THP RESULT

The formation of a visible blue line in the test “T” and control “C” regions.

13.6.2 NEGATIVE THP RESULT:

No formation of a visible blue line in the test “T” region, and a visible blue line in the control “C” region.

13.6.3 INCONCLUSIVE THP RESULT

An incomplete or questionable visible blue line in the “T” region, and a visible blue line in the control “C” region.

13.6.4 INCONCLUSIVE TEST (FAILED CASSETTE):

No visible line in the control “C” region regardless of whether or not the test “T” region exhibits a visible blue line. The test sample should be rerun, if appropriate and possible.

Note: Interpretation of the result of the test shall be documented in the case file.

Interpretation Cautions: Feces have been shown to have an inhibitory effect on the cassette, resulting in a failed test. When the presence of feces is suspected, the Urea test can be used as an additional test.

13.7 CONCLUSIONS:

13.7.1 POSITIVE UREA AND THP TESTS

Indications of urine were detected.

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13.7.2 POSITIVE THP TEST

Indications of urine were detected.

13.7.3 POSITIVE UREA TEST, BUT NEGATIVE OR UNTESTED FOR THP

Possible urine was detected, but other body fluids (such as sweat or blood) cannot be eliminated.

13.7.4 NEGATIVE UREA AND/OR THP TESTS

No urine was detected.

Note: Since a THP false negative result can occur in the presence of blood and/or vaginal secretions, when the presence of these fluids is suspected, the Urea test can be used as an additional test.

13.8 REFERENCES:

Bedrosian JL, Stolorow MD. Development of a Radial Gel Diffusion Technique for the Identification of Urea in Urine Stains. Journal of Forensic Sciences 1984; 29 (2): 601-6.

Gaensslen RE. (Urine). In, translator and editor Sourcebook in Forensic Serology, Immunology and Biochemistry. Washington, D.C.: U.S. Department of Justice 1983; p. 191-5.

Greenwald I, Gross J. The chemistry of Jaffe's Reaction for Creatinine, A Red Tautomer of Creatinine Picrate. The Journal of Biological Chemistry 1924; 59:601-12.

Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. London: Commissioner of Police of the Metropolis; 1978; p. 4-1 to 4-5.

Uritrace™ Technical Information Sheet, Uritrace™ For the Forensic and Crime Scene Identification of Urine Stains. Abacus Diagnostics Inc; 2009.

Akutsu T, Watanabe K, Sakurada K. Specificity, sensitivity, and operability of RSID™-urine for forensic identification of urine: comparison with ELISA for Tamm-Horsfall protein. J Forensic Sci. 2012 Nov;57(6):1570-3.

Independent Forensics, Developmental Validation of RSID™-Urine, <http://ifi-test.com/pdf/UrineValidation.pdf>

14.0 HAIR

14.0 PURPOSE:

To identify hair suitable for STR DNA analysis based on macroscopic and microscopic characteristics. It should be noted that the analyses described below is only a supplement to trace evidence collection and analysis by a Microanalysis Scientist (refer to section 1.8 Examination Protocol of this manual). If a hair is unsuitable for or provides insufficient results after STR DNA analysis, mitochondrial DNA analysis may still be a possibility for the unsuitable hair or the remaining shaft.

14.1 INTRODUCTION:

If hair is deemed appropriate for DNA analysis, macroscopic and microscopic observations can be used to assist in assessment.

14.2 PROCEDURE

If hairs/fibers are observed on an item during examination, but are not likely to be subjected to DNA analysis, simple documentation that they are present is sufficient (i.e. using terms such as possible hair and fiber-like).

If hairs are to be subjected to DNA analysis, examination using a stereomicroscope should be performed. It is not necessary to mount the hair for this type of examination.

14.3 INTERPRETATION

Stereomicroscopic examination should permit the following:

1. Differentiation between a hair and a fiber,
2. Differentiation between human and animal hair,
3. If a human hair is identified, if there is a root with tissue suitable for DNA analysis.
 - a. An analyst may decide to proceed for DNA analysis on a root without tissue based on the analyst's training and experience.

The results of the stereomicroscopic examination will be documented in the analyst's notes. Characteristics observed and used to determine the above categorizations should be noted and could include: Color and banding, General morphology, Medulla structure and size width, Root shape, Pigment distribution and Scale patterns/structure.

14.4 REFERENCES

Chewning, D.D., Deaver, K.L., and Christensen A.M., Persistence of Fibers on Ski Masks During Transit and Processing, Forensic Science Communications, 2008: 10(3).

Dachs, J., McNaught, I.J., and Robertson, J., The Persistence of Human Scalp Hair on Clothing Fabrics, Forensic Science International, 2003: 138(1), pp. 27-36.

Exline, D., Frequency of Pubic Hair Transfer During Sexual Intercourse, J Forensic Sci., 1998: 43, pp 505-508.

Linch, C.A., Smith, S.L., and Prahlow, J.A., Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison, J. Forensic Sci., 1998: 43 (2), pp. 305-314.

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Linch, C.A., The Ultrastructure of Tissue Attached to Telogen Hair Roots, J Forensic Sci, 2008: 53(6), pp. 1363-1366.

Moore, T.D., Identification of Dorsal Guard Hairs of Some Mammals of Wyoming, Wyoming Game and Fish Department, 1974.

Ogle, R.R. and Fox, M.J., Atlas of Human Hair – Microscopic Characteristics, CRC Press, 1999.

Pettenati, M.J. and Rao, P.N., Commentary on “Linch CA, Smith SL, Prahlow JA. Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison, J Forensic Sci. 1998; 43(2):305–14”, J Forensic Sci. 1999; 44(6): pp. 1329-1330.

15.0 WHOLE BLOOD PROCESSING

15.0 PURPOSE:

To prevent contamination of whole blood samples, and to prepare the whole blood for testing.

15.1 PROCEDURE:

THE ANALYST SHALL NEVER HAVE BLOOD TUBES FROM MORE THAN ONE SOURCE OPEN SIMULTANEOUSLY. Barrier tips shall be used to decrease the chance for contamination while making DNA stains.

THE STAINS FOR DNA SHALL BE ISOLATED FROM THE PREPARATION AREA BEFORE BLOOD TUBES FROM ANOTHER SOURCE OF BLOOD ARE OPENED. This isolation can be done by moving the prepared stains to another area, by covering the prepared stains, or by placing a barrier between the prepared stains and the blood processing area.

15.2 STAINS FOR DNA

If enough blood is present, deposit on the paper DNA cards several aliquots of whole blood. The cards should be labeled with case number, name of the blood source, date the stains are made, and the initials of the individual making the stain. Additionally, if the blood is from a tube other than a lavender-top Vacutainer®-style tube, a notation indicating the deviation (i.e. "gray-top") may also be written on the card. If using some bodily substance other than blood (i.e. liver tissue), the body substance may be noted on the card.

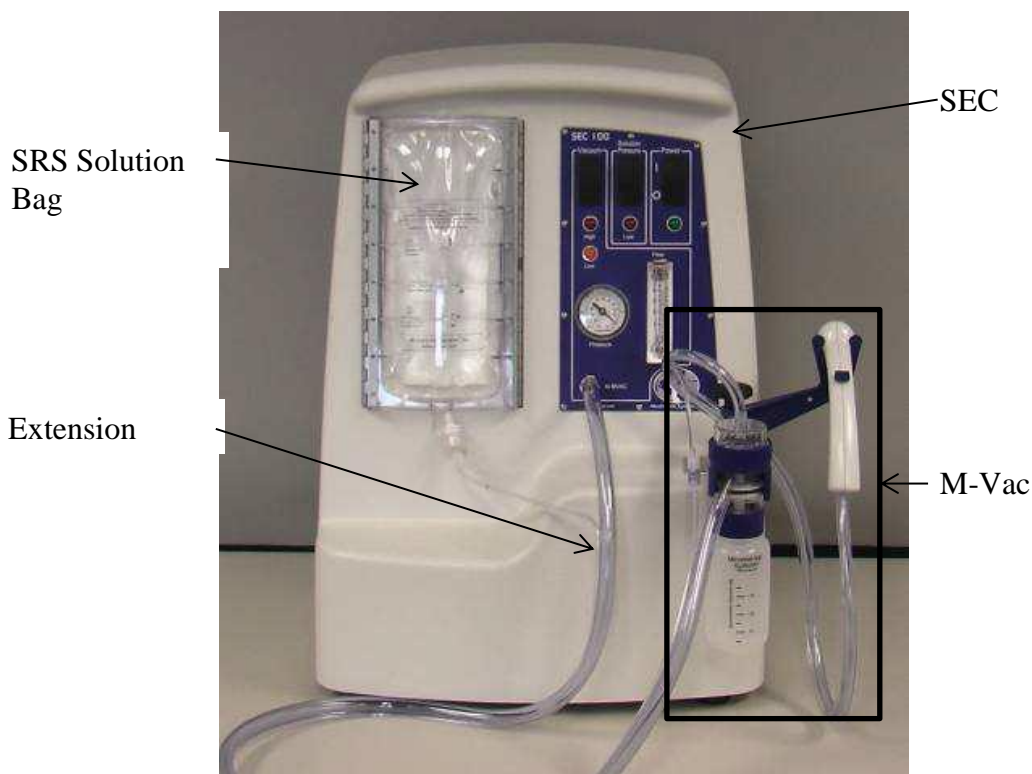
If the blood (or other bodily substance) is not in a liquid form, such as coagulated blood in a red-top tube, then some of the solid material should be placed on and worked into the card using an applicator stick, stirring rod, or other clean utensil.

Prepared reference cards shall be dried. The reference card may be used in a DNA extraction procedure. When the testing has been completed on the case, the card shall be returned to the submitting agency.

Alternatively, a small aliquot (~3-5µl) of liquid blood may be placed directly into a tube and extracted for DNA content; however, a reference card should still be made for long-term storage.

16.0 MSI M-VAC® SYSTEM

The MSI M-Vac System is for the wet-vacuum collection of cellular material from forensic samples where limited amounts of DNA may be present and where other collection techniques may not be successful. The MSI M-Vac System consists of a Support Equipment Case (SEC, which provides the power and vacuum), extension tubing to the M-Vac, a sterile surface rinse solution (SRS) bag, and the M-Vac (see below). Each sample collection requires a new M-Vac (see System Set-Up, Sample Collection, & Removing/Replacing M-Vac) where the cellular sample is suspended in a collection bottle included with the M-Vac (see Removing/Replacing Sample Collection Bottle). The SRS bag provides the buffer enabling the suspension of cellular material and is required for sample collection; the SRS bag can remain on the SEC for multiple collection events and should be replaced as needed for sample collection (see Replacing SRS Bag). For some collection events, multiple collection bottles or SRS bags may be needed. The cellular sample in the collection bottle must be filtered prior to DNA extraction (see Filtering a Sample).



16.1 SYSTEM SET-UP:

1. Turn ON the Support Equipment Case (SEC) power switch.
2. Remove over wrap from the Surface Rinse Solution (SRS) bag and hang the bag on the solution door.
3. Prepare the M-Vac for use
 - a. Remove the M-Vac and tighten the lid (tighten, release, tighten)
 - b. Turn OFF the solution switch on sampling head by pulling back
 - c. Place separation unit and sampling head in holder on SEC
4. Open extension tubing. Attach the solution line fitting to the M-Vac fitting on the M-Vac. Lightly attach the vacuum tubing.
5. Aseptically break tip off the SRS bag. Connect spiked fitting of the tubing to the bag port. To connect, push and twist the fittings together until fully seated.

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6. Close the door until it is locked shut by the hinged latch. Turn Solution Pressurization switch ON. (It is pressurized when the low pressurization indicator light turns OFF.)
7. Connect vacuum side of tubing to SEC by slipping the quick-connect fitting into the vacuum port on SEC labeled "to MVAC."

16.2 SAMPLE COLLECTION:

1. Turn the Vacuum switch of the SEC to ON.
2. With the vacuum pump ON, retighten the lid on the Separation Unit.
3. Place the sampling head against the surface to be sampled. (Try to keep all the flexible feet in light contact with the surface while sampling.)
4. Turn ON and OFF the solution by toggling the switch on the sampling head as needed.
5. When the sample has been taken, or if additional bottles are needed to complete the sample, turn the Vacuum switch on the SEC to OFF. Remove the sample collection bottle.

16.3 REMOVING/REPLACING M-VAC:

1. Turn OFF the vacuum.
2. Turn OFF the Solution Pressurization (optional).
3. Pull vacuum tubing off hose barb on the side of the Separation Unit.
4. Disconnect the solution line at the Separation Unit by unthreading the M-Vac fitting from the check valve.
5. Remove the Separation Unit and Sampling Head from their holders and discard.
6. Open and connect a new M-Vac (optional)

16.4 REPLACING SRS BAG:

1. Depressurize the Solution Pressurization chamber by turning the Solution Pressurization switch to OFF.
2. Open Solution Pressurization Chamber and remove used SRS bag from hook.
3. Disconnect spiked fitting on solution line from threaded SRS bag port fitting. Discard old SRS bag.
4. Connect a new SRS bag (see "System Set-up")

16.5 REMOVING/REPLACING SAMPLE COLLECTION BOTTLE:

1. Turn Vacuum switch to OFF.
2. Unscrew the bottle from the Separation Unit.
3. Place a lid on the removed bottle and tighten.
4. If the M-Vac is going to be used to continue sampling an area or to take another sample, screw a new bottle on the Separation Unit.

16.6 FILTERING A SAMPLE:

1. Attach the M-Vac vacuum tubing to the vacuum filter.
2. Support the vacuum filter in the upright position during processing.
3. Turn ON the vacuum on the SEC.
4. Swirl the sample in the bottle and then slowly pour into the vacuum filter funnel.
5. Continue vacuuming until all the solution has passed through the filter.
6. If desired, turn OFF the vacuum, remove the collection bottle on the bottom of the filter, pour the filtered water back into the sample bottle, swish and re-filtrate after the vacuum filter is reassembled and the vacuum is turned ON.
7. If desired, the vacuum filter funnel can be rinsed with DI water at the end of filtering to rinse the sides of the funnel.
8. Discard the filtered solution.

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9. Handle the filter/filter funnel apparatus with standard evidence handling practices (if desired, the filter can be stored and dried while in the housing for later processing).
10. Using a sterile scalpel remove and process the entire filter for DNA using an appropriate extraction method.

16.7 M-VAC SYSTEM MAINTENANCE:

1. Day of use:
 - a. Ensure the switches are OFF on the Vacuum, Solution Pressure, and Power.
 - b. If the vacuum tubing or solution will be used again, leave an M-Vac (sampling head and separation unit) attached and in the OFF position.
 - c. In the event of contamination, wipe down SEC with a suitable disinfectant.
2. Periodic (schedule may vary based on usage):
 - a. Check the liquid trap and empty if needed.
 - b. Inspect the ventilation filter, and if dirty, clean with a mild soap or replace if needed.
 - c. Replace the Vacuum Exhaust HEPA Filter anytime there is reason to believe contamination is present in the vacuum exhaust.

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17.0 SAFETY

During daily serological routines, the analyst is likely to come into contact with hazardous and potentially hazardous chemicals. Skin contact with any chemical shall be avoided. Use personal protection equipment (i.e. gloves, lab coat mask) as required. Also, adequate ventilation shall be used when handling chemicals, especially liquids. Material and Safety Data Sheets (MSDS) shall be readily available to reference. Questions not answered by the MSDS regarding the safe use of any chemical shall be directed to the laboratory safety officer.

Below is a list of commonly used chemicals in serology, which should be handled carefully.

Chronic exposure to any chemical may increase the potential for Carcinogenic, Teratogenic, Mutagenic, and Reproductive hazards.

17.0 ACETIC ACID:

Combustible.

Causes severe burns, harmful in contact with skin.

17.1 ACETONE:

Flammable.

Irritating to eyes and skin.

Inhalation may cause drowsiness and dizziness.

17.2 AMMONIUM HYDROXIDE:

Corrosive.

Harmful if swallowed, causes burns.

Dangerous for the environment.

17.3 AMMONIUM NITRATE:

OXIDIZER-contact with combustible material may cause fire.

Irritating to eyes, respiratory system, and skin.

17.4 BRILLIANT BLUE R (COOMASSIE BLUE):

Contact and inhalation hazard.

17.5 BROMOTHYMOL BLUE:

Contact and inhalation hazard.

17.6 DIANISIDINE TETRAZOTIZED (FAST BLUE B):

Toxic.

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May cause cancer.

Harmful if swallowed.

17.7 INDIGO CARMINE:

Harmful if swallowed

17.8 MERCURIC CHLORIDE:

Highly toxic.

Causes burns.

Very toxic in contact with skin or if swallowed.

Rapidly absorbed through skin.

17.9 METHANOL:

Flammable.

Toxic by inhalation, in contact with skin, and by ingestion.

Irritating to the eyes and skin.

17.10 NUCLEAR FAST RED:

Irritant to eyes, respiratory system, and skin.

17.11 PHENOLPHTHALEIN:

Possible carcinogen.

17.12 PHOSPHORIC ACID:

Corrosive.

Causes burns.

Harmful if swallowed.

17.13 POTASSIUM HYDROXIDE:

Corrosive.

Causes severe burns.

Harmful if swallowed.

17.14 PYRIDINE:

Flammable.

Harmful by inhalation, in contact with skin, and if swallowed.

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Risk of serious eye damage.

Possible carcinogen.

17.15 SODIUM HYDROXIDE:

Corrosive.

Causes severe burns.

Exothermic in contact with skin.

17.16 SODIUM A-NAPHTHYL ACID PHOSPHATE:

Irritating to eyes, respiratory system, and skin.

17.17 SODIUM PHOSPHATE, MONOBASIC:

May cause skin irritation.

17.18 SODIUM PHOSPHATE, DIBASIC:

May cause skin irritation.

17.19 ZINC CHLORIDE:

Corrosive.

Causes burns.

Harmful if swallowed.

Dangerous to the environment.

The chemicals which require special handling are indicated in the "Reagent Preparation" section of this manual with a designation of (HAZMAT).

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18.0 REAGENT PREPARATION

Use reagent grade chemicals unless otherwise noted. Pre-made, reagent grade chemicals can be substituted for many of the recipes below. Prepare all solutions using deionized water (dH₂O). Wear appropriate personal protection equipment such as gloves and follow safety recommendations provided by manufacturer for handling chemicals. Comply with any and all laws, regulations, or orders with respect to the disposal of any hazardous or toxic chemical, material, substance or waste. Store all reagents at room temperature unless otherwise noted. Reagents are prepared as follows:

18.0 PHOSPHATE BUFFERED SALINE (PBS)

PBS shall be purchased from a commercial supplier and shall have a formulation of approximately pH 7.4, 137 mM salt and at minimum 10 mM PO₄. PBS can be purchased in a 1X or 10X solution.

18.0.1 HAZMAT

PBS is a skin, eye, and respiratory irritant.

18.1 PHENOLPHTHALEIN

18.2 PHENOLPHTHALIN STOCK SOLUTION

2 g Phenolphthalein CAS No. 77-09-8 (HAZMAT)

20 g Potassium hydroxide (HAZMAT)

100 ml dH₂O

The mixture is refluxed with 20 grams of powdered zinc (approximately two hours) until the solution becomes colorless. The stock solution shall be stored in an appropriate container in a cold dark place (i.e. refrigerator) with some zinc added to keep it in the reduced form.

Phenolphthalin Stock Solution (alternate method)

2g Phenolphthalin CAS No. 81-90-3 (HAZMAT)

20 g Potassium hydroxide (HAZMAT)

100 ml dH₂O

Place 20g of potassium hydroxide pellets into a 500ml amber reagent bottle with zinc turnings and 2g of phenolphthalin powder. Add 100ml dH₂O to the contents of the bottle and swirl until dissolved. The stock solution shall be stored in an appropriate container in a cold dark place (i.e. refrigerator) with some zinc added to keep it in the reduced form

This reagent expires 3 years after its preparation. A lot number, which will be the six digit expiration date, shall be assigned and recorded on the bottle and in the Reagent Log.

18.2.1 HAZMAT

Phenolphthalein is a possible carcinogen.

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Potassium hydroxide is corrosive and will cause severe burns when contacting skin, eyes, and mucous membranes. Harmful if swallowed.

18.2.2 DISPOSAL

Zinc may be stored until it is picked up by a chemical waste removal company. Zinc shall not be disposed of in a waste paper basket! All waste shall be disposed of according to local regulations.

18.3 PHENOLPHTHALIN WORKING SOLUTION

10 ml Phenolphthalin stock solution

40 ml EtOH

Combine 10 ml of phenolphthalin stock solution and 40 ml ethanol. The prepared working solution shall be stored in an appropriate container with some zinc to keep it in the reduced form and may be stored in the refrigerator or at room temperature.

The lot number, which is the lot number of the stock solution followed by a unique identifier (i.e. - A, -B, -1, -2), shall be recorded on the bottle. Before use on casework samples, working reagent shall be QC checked and recorded in the Reagent Log. Working reagent will also be tested with a positive blood control and negative control each day of use. (The results of these tests shall be recorded in the case notes).

18.3.1 DISPOSAL

All waste shall be disposed of according to local regulations.

Zinc may be stored until it is picked up by a chemical waste removal company. Zinc shall not be disposed of in a waste paper basket.

18.4 TAKAYAMA

18.4.1 TAKAYAMA REAGENT

5 ml Saturated glucose solution

5 ml 10% NaOH (HAZMAT)

5 ml Pyridine (HAZMAT)

10 ml dH₂O

The stock solution shall be stored in an appropriate container in a cold dark place (i.e. refrigerator). This reagent expires 6 months after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log. Before use on the casework samples, the reagent shall be tested with the documentation positive blood control each day of use. The results of these tests shall be recorded in the case notes.

18.4.2 HAZMAT

NaOH is corrosive and will cause severe burns in contact with the skin, eyes, and mucous membranes. It also can react violently with acids.

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Pyridine is a flammable liquid. Harmful by inhalation, ingestion, and skin contact. Exposure may result in serious eye damage. Pyridine is a possible carcinogen.

18.4.3 DISPOSAL

All waste shall be disposed of according to local regulations.

18.5 OUCHTERLONY

0.2% Coomassie Blue Stain

1.0 g Brilliant Blue (R250) (HAZMAT)

500 ml Destain solution

Combine reagents and stir until dissolved.

This reagent expires 3 years after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

18.5.1 HAZMAT

Brilliant Blue is an irritant. Avoid skin contact and inhalation of fumes.

18.5.2 DISPOSAL

All waste shall be disposed of according to local regulations.

18.6 DESTAIN SOLUTION

50 volumes MeOH (HAZMAT)

10 volumes Glacial acetic acid (HAZMAT)

50 volumes dH₂O

Combine all reagents.

This reagent expires 3 years after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

18.6.1 HAZMAT

Methanol is a flammable liquid. Toxic by ingestion, inhalation, and skin contact. It is a cumulative poison. It is an eye and skin irritant.

Glacial acetic acid is combustible. It will cause burns. Avoid contact with the skin and mucous membranes.

18.6.2 DISPOSAL

All waste shall be disposed of according to local regulations.

18.7 AGAR PLATES

0.5 g Oxoid ion agar

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90 ml Isotonic saline

10 ml 1:1000 Merthiolate solution

Boil Oxoid ion agar in isotonic saline to melt, cool gel to 50°-60° C, add 1:1000 merthiolate solution, and pour. Wells may be cut after the gel has set.

*Gels are also commercially available.

18.7.1 DISPOSAL

Gels shall be disposed of in biohazard waste containers.

18.8 ACID PHOSPHATASE

18.9 ACID PHOSPHATASE STOCK SOLUTION (A)

1 g Fast Blue B (HAZMAT)

20 g Sodium acetate, trihydrate

10 ml Glacial acetic acid (HAZMAT)

100 ml dH₂O

Combine and store in a cool dark place (i.e. refrigerated).

This reagent expires 6 months after its preparation. Each lot must be recorded in the reagent log. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle.

18.9.1 HAZMAT

Fast Blue B is a suspected carcinogen. Avoid ingestion and inhalation.

Glacial acetic acid is combustible and can cause severe burns to the skin, eyes, and mucous membranes. Avoid inhalation.

18.9.2 DISPOSAL

All waste shall be disposed of according to local regulations.

18.10 ACID PHOSPHATASE STOCK SOLUTION (B)

0.4 g Sodium α -naphthyl acid phosphate (HAZMAT)

5 ml dH₂O

Combine and store in a cool dark place (i.e. refrigerated).

This reagent expires 6 months after its preparation. Each lot must be recorded in the reagent log. A lot number, comprised of the six digit expiration date shall be recorded on the bottle.

18.10.1 HAZMAT

Sodium α -naphthyl acid phosphate is an irritant. Avoid inhalation and contact with skin and eyes.

18.10.2 DISPOSAL

All waste shall be disposed of according to local regulations.

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18.11 ACID PHOSPHATASE WORKING SOLUTION

Brentamine reagent, also known as Acid Phosphatase Working Solution

10 ml Solution A

1 ml Solution B

89 ml dH₂O

Combine and store in a cool dark place (i.e. refrigerated).

The expiration date (2 weeks after preparation) shall be recorded on the bottle. Before use on casework samples, working reagent shall be QC checked and recorded in the Reagent Log. Working reagent will also be tested with a documented semen control each day of use. A negative control shall also be tested. The results of these tests shall be recorded in case notes.

Alternative preparation method:

AP Spot Test (SERI) may be prepared following the manufacturer's directions.

Per manufacturer's instructions, the AP Spot reagent will be made fresh each day of use.

Before use on casework samples, working reagent shall be QC checked. Working reagent will also be tested with a documented semen control each day of use. A negative control shall also be tested. The results of these tests shall be recorded in case notes.

18.11.1 DISPOSAL

All waste shall be disposed of according to local regulations.

18.12 SPERM SEARCH

It is preferable to obtain Stains A and B of the Christmas Tree Stain procedure commercially.

Mounting media (i.e. Permout or Cytoseal) can be used beyond its commercially designated expiration date as long as its functional properties remain intact.

18.13 PHADEBAS®

Phadebas® Paper (purchased commercially)

Distilled H₂O (do not use de-ionized water instead of distilled)

Note. Distilled water will be given a six digit lot number depicting a three-year expiration date from receipt if an expiration date is not already supplied by the vendor and will be recorded in the chemical inventory log.

Store Phadebas® paper at room temperature. A lot number, comprised of the six digit expiration date supplied by the vendor, shall be recorded on the packaging. Prior to use in casework, each lot of paper must be QC checked using a positive neat saliva control swab and recorded in the Reagent Log.

All waste shall be disposed of according to local regulations.

18.14 FECAL

Urobilinogen

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Alcoholic Mercuric Chloride6.78g HgCl₂ (HAZMAT)

25ml MeOH (HAZMAT)

Combine and store in a cool dark place (i.e. refrigerator). This reagent expires 3 years after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

18.15 ALCOHOLIC ZINC CHLORIDE3.407g ZnCl₂ (HAZMAT)

25ml MeOH (HAZMAT)

Combine and store in a cool dark place (i.e. refrigerator). This reagent expires 3 years after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

18.15.1 HAZMAT

Mercuric chloride is highly toxic and causes burns. Mercuric chloride is very toxic by ingestion, skin contact, and inhalation. Mercuric chloride is rapidly absorbed through the skin. It is a cumulative poison.

Zinc chloride is corrosive and causes severe burns to skin and tissue. Harmful if swallowed. It is a possible mutagen, teratogen, and carcinogen.

Methanol is a flammable liquid. Toxic by ingestion, inhalation, and skin contact. It is a cumulative poison. It is an eye and skin irritant.

18.15.2 DISPOSAL

Waste containing mercuric chloride shall be disposed of as hazardous waste. All other waste shall be disposed of according to local regulations.

18.16 0.1 N SODIUM HYDROXIDE

0.4g NaOH (HAZMAT)

100 ml dH₂O

10% Sodium Hydroxide

10g NaOH (HAZMAT)

100ml dH₂O

Combine and mix. This reagent expires 3 years after its preparation. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle. The reagent documentation shall be recorded in the reagent log.

Alternatively, a more concentrated stock solution can be made with a lot number assigned, and preparation or expiry date recorded on the bottle. As required an appropriate dilution can be prepared with the preparation date recorded on the bottle.

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18.16.1 HAZMAT

NaOH is poisonous by ingestion and intraperitoneal routes. It is a skin and eye irritant. It has marked corrosive action on all body tissue. It can react violently with acids.

18.16.2 DISPOSAL

All waste shall be disposed of according to local regulations.

18.17 BROMOTHYMOL BLUE (BTB)

150mg BTB (HAZMAT)

10.0ml dH₂O

50µl 1:10 Phosphoric acid (HAZMAT)

Dissolve BTB in dH₂O with the addition of 1:10 phosphoric acid.

This reagent expires when the bromothymol blue stock expires (or three years after preparation if no expiry date is provided by the manufacturer). Each lot must be recorded in the reagent log. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle.

18.17.1 HAZMAT

Bromothymol blue may be harmful by inhalation, ingestion, or skin absorption. It may cause eye irritation and skin irritation.

Phosphoric acid is corrosive and may cause burns to the skin, eyes, and mucous membranes. Harmful if swallowed.

18.17.2 DISPOSAL

All waste shall be disposed of according to local regulations.

18.18 UREASE

25mg Urease

10ml dH₂O

0.1 N NaOH (HAZMAT)

Dissolve urease in dH₂O and adjust the pH to 5.8 to 6.0 with 0.1N NaOH (or HCl as appropriate).

This reagent expires 1 week after its preparation. Each lot must be recorded in the Reagent log. A lot number, comprised of the six digit expiration date, shall be recorded on the bottle.

18.18.1 HAZMAT

NaOH is corrosive and will cause severe burns in contact with the skin, eyes, and mucous membranes. It also can react violently with acids.

18.18.2 DISPOSAL

All waste shall be disposed of according to local regulations.

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18.18.3 UREASE GEL PLATES

Plates are best when used fresh (day of use). Dissolve 2.0 grams of type I agarose in 100 ml of dH₂O, with heating (>60° C). To the warm agarose solution, add 1.0 ml of the BTB solution. While stirring, add 0.01N NaOH drop-wise, until the pH is approximately 6 (corresponding to a green color).

Pour half of the agarose into a separate beaker, and when the temperature of the agarose has dropped to 60° C, add 2.5 ml of the urease solution to one of the agarose aliquots. Aliquot equal amounts of the urease containing agarose into each of several plastic Petri dishes. Aliquot equal amounts of regular agarose into a corresponding number of plates. Label the dishes appropriately. Allow the gels to cool and solidify. Punch holes in the gels with a gel punch connected to an aspirator, leaving at least 1.5 cm between holes.

Note: Plates may be used up to one week after preparation, if stored refrigerated.

18.18.4 DISPOSAL

Gels shall be disposed of in biohazard waste containers.

19.0 GLOSSARY

19.0 ACID PHOSPHATASE (AP)

An enzyme found at elevated levels in semen and lower levels in some other body fluids.

19.1 AMYLASE

An enzyme found at elevated levels in saliva and lower levels in some other body fluids.

19.2 CONFIRMATORY TEST

One or more procedures, the result(s) of which justifies a conclusion of identification.

19.3 ALTERNATE LIGHT SOURCE (ALS)

An instrument which delivers a high intensity light of specific wavelength. Different types of evidence, such as semen or fibers, may fluoresce during exposure to this light. Other types of evidence, such as bloodstains, may absorb light.

19.4 HEMATRACE® TEST

A test for hemoglobin; confirmatory for blood and indicative of human origin.

19.5 NUCLEATED EPITHELIAL CELLS

Nucleated cells which form the lining of body cavities (i.e. mouth, vagina) and the outermost layers of skin. These cells are shed regularly from the human body.

19.6 P30

A protein used to identify semen.

19.7 P30 TEST

A test for human p30.

19.8 PHENOLPHTHALEIN

A presumptive test for the presence of blood.

19.9 PRESUMPTIVE TEST

A preliminary test to ascertain the presence of a biological substance.

19.10 OUCHTERLONY

A test used to determine a species of origin.

19.11 SPERMATOZOA

The male reproductive cells (identified microscopically).

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19.12 TAKAYAMA

A confirmatory test for blood.

19.13 UREA

A major constituent of urine.

19.14 UREASE

An enzyme present in urine

19.15 UROBILINOGEN

A substance excreted in feces.

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20.0 REVISIONS

Biochemistry Procedures Manual version 08-2006

Extensive update and re-organization of manual and addition of Phadebas® Paper amylase testing method

Table of Contents version 03-2007

Addition of Revisions section

Reagent Preparation version 03-2007

Acid Phosphatase working solution wording of lot number and preparation or expiration date changed to just preparation date recorded on bottle. Allowance added for using a more concentrated NaOH to make dilutions of needed working solutions.

RSID® Saliva Cards version
06-2007

New method added.

Biochemistry Procedures Manual version 09-2008

Converted to CLD ISO format, p30 procedure update, reagent prep update, HemeTrace update, Introduction update.

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